

CLAIMS

1. A method of selecting and / or identifying one or more protein affinity ligand's that bind to one or more proteins of interest comprising the steps of:
- (A) obtaining a real or theoretical peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation of the one or more proteins by either:
- i. Subjecting the one or more proteins to peptide mass fingerprinting or other mass spectrometry based characterisation or other protein characterisation; or
 - ii. Predicting the peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation from known data;
- (B) utilising the one or more proteins either individually or as a mixture to:
- i. Generate one or more antibodies thereto by immunisation ;and/or
 - ii. Select, using a single or multiple rounds of binding, one or more protein affinity ligands thereto;
- (C) screening the one or more antibodies generated in step (B)(i) and/or the one or more protein affinity ligands selected in step (B)(ii) by:
- i. adding the one or more proteins individually or as a mixture of proteins to the one or more antibodies generated in step (B)(i) or the one or more protein affinity ligands selected in step (B)(ii), each antibody or protein affinity ligand being used individually, and

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ii. after removing any proteins which have not bound, eluting the at least one protein that has bound;

(D) subjecting the at least one eluted protein to peptide mass fingerprinting and / or other mass spectrometry based characterisation and/or other protein characterisation ; and

(E) by comparing the peptide mass fingerprints or other mass spectrometry based characterisation or other protein characterisation obtained in steps (A) and (D) selecting and/or identifying the at least one protein affinity ligand that binds to the one or more proteins of interest.

2. A method as claimed in claim 1 wherein the one or more proteins of interest are resolved by 2D electrophoresis.

3. A method as claimed in claims 1 or 2 wherein between steps (B) and (C) the antibodies obtained in step (B)(i.) are cloned.

4. A method as claimed in any of the preceding claims wherein the one or more proteins of interest are present in a mixture of proteins.

5. A method as claimed in any of the preceding claims wherein the method is a shotgun method for selecting and identifying protein affinity ligands to a plurality of proteins.

6. A method as claimed in any of the preceding claims wherein the other mass spectrometry based characterisation includes acquisition of sequence tag data.

7. A method as claimed in any of the preceding claims wherein the antibodies generated in step (B)(i.) are immobilised on a support comprising

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nitrocellulose or PVDF.

8. A method as claimed in claim 7 wherein the support upon which the antibodies are immobilised are treated with an oligosaccharide or polyvinylpyrrolidone solution to block any remaining binding sites.

9. A method as claimed in claim 8 wherein the oligosaccharide is ficoll.

10. A method as claimed in any of claims 7 to 9 wherein the eluting agent is a volatile reagent.

11. A method as claimed in claim 10 wherein the volatile reagent is formic acid.

12. A method of generating monoclonal antibodies to one or more targeted proteins comprising the steps of:

- (a) resolving a complex protein mixture;
- (b) subjecting the resolved protein(s) to peptide mass fingerprinting to obtain a peptide mass profile or obtain a theoretical peptide mass profile;
- (c) utilising one or more of the resolved proteins to generate one or more monoclonal antibodies thereto;
- (d) adding the or another complex protein mixture to the one or more monoclonal antibodies generated in Step (c), to select those proteins which bind the one or more monoclonal antibodies, and subjecting the selected protein(s) to peptide mass fingerprinting to obtain a peptide mass profile;
- (e) comparing the peptide mass profiles obtained in steps (b) and (d); and
- (f) selecting one or more hybridoma clones of interest.

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13. A method of generating an antibody library comprising the steps of:

- (a) resolving a complex protein mixture and subjecting the resolved protein(s) to peptide mass finger printing to obtain a peptide mass profile; or
- (b) obtaining a theoretical peptide mass profile for a protein which is sought;
- (c) utilising the or another complex protein mixture to generate one or more monoclonal antibodies thereto;
- (d) adding the or the other complex protein mixture to the one or more monoclonal antibodies generated in Step (c) to select those proteins which bind the one or more monoclonal antibodies, and subjecting the selected protein(s) to peptide mass fingerprinting to obtain a peptide mass profile;
- (e) comparing the peptide mass profiles obtained in steps(a or b) and (d);
- and
- (f) identifying the monoclonal antibodies of potential interest for a monoclonal antibody library.

14. A process for selecting desired members of an affinity ligand library comprising the steps of:

- (a) resolving a complex protein mixture and subjecting the resolved protein(s) to peptide mass finger printing to obtain a peptide mass profile; or
- (b) obtaining a theoretical peptide mass profile for a protein which is sought;
- (c) utilising one or more of the resolved proteins to select one or more affinity ligands from a library;
- (d) adding the or another complex protein mixture to the on or more affinity

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ligands generated in step (c) to select those proteins which bind the one or more affinity ligands, and subjecting the selected protein(s) to peptide mass fingerprinting to obtain a peptide mass profile;

(e) comparing the peptide mass profiles obtained in steps (a or b) and (d);

and

(f) selecting one or more affinity ligands of interest.

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15. A method of screening an affinity ligand to a protein characterised in that the affinity ligand is generated or selected using an impure protein or a complex protein mixture and then identified by comparing a peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation obtained from the protein/proteins for which it is specific with that of a peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation which is theoretical for said protein/proteins or is obtained from the impure protein or complex protein mixture.

16. A method of selecting an antibody or other protein affinity ligand specific to a given peptide characterised in that the antibody or other protein affinity ligand is selected by comparing a peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation of the protein/proteins released from the antibody or other protein affinity ligand to which it binds with a peptide mass fingerprint or other mass spectrometry based characterisation or other protein characterisation which is theoretical for said protein/proteins or is obtained from the known protein.

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18. A method as claimed in any of the preceding claims further comprising the use of automated well plate handling technology and automated high-throughput mass spectrometry.

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